Insulin resistance and inflammation predict kinetic body weight changes in response to dietary weight loss and maintenance in overweight and obese subjects by using a Bayesian network approach¹–⁴


ABSTRACT

Background: The ability to identify obese subjects who will lose weight in response to energy restriction is an important strategy in obesity treatment.

Objective: We aimed to identify obese subjects who would lose weight and maintain weight loss through 6 wk of energy restriction and 6 wk of weight maintenance.

Design: Fifty obese or overweight subjects underwent a 6-wk energy-restricted, high-protein diet followed by another 6 wk of weight maintenance. Network modeling by using combined biological, gut microbiota, and environmental factors was performed to identify predictors of weight trajectories.

Results: On the basis of body weight trajectories, 3 subject clusters were identified. Clusters A and B lost more weight during energy restriction. During the stabilization phase, cluster A continued to lose weight, whereas cluster B remained stable. Cluster C lost less and rapidly regained weight during the stabilization period. At baseline, cluster C had the highest plasma insulin, interleukin (IL)-6, adipose tissue inflammation (HAM56+ cells), and Lactobacillus/Leucostoc/Pediococcus numbers in fecal samples. Weight regain after energy restriction correlated positively with insulin resistance (homeostasis model assessment of insulin resistance: \( r = 0.5, P = 0.0002 \)) and inflammatory markers (IL-6; \( r = 0.43, P = 0.002 \)) at baseline. The Bayesian network identified plasma insulin, IL-6, leukocyte number, and adipose tissue (HAM56) at baseline as predictors that were sufficient to characterize the 3 clusters. The prediction accuracy reached 75.5%.

Conclusion: The resistance to weight loss and proneness to weight regain could be predicted by the combination of high plasma insulin and inflammatory markers before dietary intervention. This trial was registered at clinicaltrials.gov as NCT01314690. Am J Clin Nutr 2013;98:1385–94.

INTRODUCTION

Obesity is a major health problem that is dramatically increasing worldwide and is associated with comorbidities. Of different therapeutic approaches, a dietary intervention remains the method of choice (1). However, interindividual variability in the response to dietary changes and weight regain are major challenges (2), with only ~20% of overweight individuals being successful at long-term weight loss (3) as illustrated by the results of a European study that included 8 centers [the Diet, Obesity and Genes (Diogenes) Study]. In 548 subjects in the study, only 124 individuals (22.5%) maintained a stable weight after weight loss (4). Thus, to improve the clinical care of obese subjects, it is essential to identify individuals who will significantly lose weight after energy restriction and maintain stable weight afterward.

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4 Hypocaloric supplements for the whole period of the study were offered by KOT-Ceprodi Laboratory (Florence Massiera, Réginald Allouche) Paris, France.
Over the past 20 years, researchers have attempted to find different predictors of weight loss induced by energy restriction and others associated with weight maintenance. Environmental, psychological, systemic, and genetic factors have been implicated. Baseline high adiponectin concentrations (5) and the degree of weight loss during the first week of energy restriction (6) have been associated with successful weight loss. Predictors of successful weight maintenance have been numerous factors such as the resting energy expenditure (REE) (7), plasma leptin (5), and angiotensin I–converting enzyme concentrations (8). The messenger RNA expression of subcutaneous adipose tissue (scAT) genes has also been associated with weight loss (9) or weight maintenance. Subjects who regained weight after diet-induced weight loss showed an overexpression of genes involved in cellular growth and proliferation, and subjects with continuing weight loss had an overexpression of genes grouped in the mitochondrial oxidative phosphorylation functions (10). Generally, the degree of prediction accuracy currently achieved with a gene-expression snapshot appears insufficient for clinical use.

Because of the complexity of obesity, additional markers should be considered to find predictors of the best weight trajectory. In human and animal models, direct or indirect relations between gut microbiota and obesity have been widely discussed (11). To our knowledge, no study has evaluated if gut-microbiota components could be valuable predictors of weight variation in response to a dietary intervention. Most previous studies focused on factors that predicted either the weight-loss or weight-regain period, and none of the studies considered the individual kinetic profile during a whole dietary intervention.

Statistical methods used to find predictors have been mostly focused on Spearman’s correlations and multivariate linear regression models, which barely addressed the complex and conditional relations in variables. A combination of gut-microbiota components and physical and biological variants with a suitable framework could improve the overall prediction. We proposed a graphical Bayesian network (BN) framework for predicting weight loss and regain. This method has been used for disease prediction (12) and predicting physical characteristics such as eye color by using genetic data (13). It has never been used in the context of predicting weight loss in dietary interventions.

This study was conducted to identify predictive markers of the best weight profile in a kinetic dietary study with a weight-loss phase followed by a weight-maintenance period. Tested variables included quantitative and qualitative food items and metabolic and inflammatory variables, as well as components of gut microbiota. The relevance of predictors was tested by using a BN approach.

**SUBJECTS AND METHODS**

Fifty overweight/obese [25–65 y old; BMI (in kg/m²): 25–38] but otherwise healthy individuals (8 men and 42 women) were recruited for a 12-wk dietary intervention at the Research Center on Human Nutrition (Pitié-Salpêtrière Hospital). One subject dropped out for personal reasons. Subjects with diabetes, in-inflammatory or infectious diseases, and gastrointestinal, hepatic, renal, or cardiac problems were excluded. None of the participants was taking chronic treatments or had been involved in weight-loss programs in the preceding 12 mo. No antibiotics were taken 2 mo before or during the study. Forty-nine subjects completed the study. The sample size was calculated by fixing the probability of type 1 error at 0.05 and the type 2 error at 0.10 for changes in body fat mass (−5% to −7%). Each subject provided written informed consent. The Ethical Committee of Hotel-Dieu Hospital approved the protocol.

Subjects were submitted to a 6-wk hypocaloric diet (1200 kcal/d for women and 1500 kcal/d for men) followed by a second period of 6 wk of a weight-maintenance diet. Because total energy expenditure might be greater in men than women, men were prescribed a higher caloric intake to reach the same body weight loss as women in the first phase. Because the recommended daily calories in adults with habitual physical activities for the majority of the French population are 2200 kcal for women and 20% more for men (2640 kcal) (14), we kept the same ratio between men and women (1200 kcal for women; 1500 for men; +20%).

The hypocaloric diet was high in protein and rich in fiber, with low–glycemic index carbohydrates (35% protein, 25% fat, and 40% carbohydrate). This diet consisted of typically consumed foods with the addition of 4 dietary supplements daily (60–75 kcal) as described previously (15). The 6-wk weight-maintenance diet was prescribed individually by a diettian and was, on average, 20–30% more than the REE as a function of each subject’s REE and physical activity levels (16) as described previously (17). At the beginning (day 0) and after 6 and 12 wk, participants underwent a series of tests after an overnight fast.

**Adipocyte morphology and immunohistochemical analysis**

One-half hour after arrival, a sample of scAT was obtained by needle biopsy from the periumbilical area under local anesthesia (1% xylocaine). A fresh aliquot was collected to measure the adipocyte diameter, as described previously (15), and another aliquot was stored overnight at 4°C in 4% paraformaldehyde and embedded in paraffin for immunohistochemical detection. We used an anti-HAM56 antibody (Dako Cytomation) to target macrophages in scAT by using the avidin-biotin-peroxidase method. Two independent investigators counted the number of positively stained cells (18).

**Insulin sensitivity**

The estimation of pancreatic β cell function (insulin secretion) and insulin sensitivity were calculated by using homeostasis model assessment with homeostasis model assessment/Continuous Infusion of Glucose with Model Assessment software (19). Quantitative insulin-sensitivity check index (QUICKI) values were determined by using a mathematical transformation of fasting blood glucose and insulin measurements. Disse and McAule (20) indices were estimated as described previously (20). The AUC during an oral-glucose-tolerance test (OGTT) was also calculated.

**Body composition**

Body fat and fat-free mass distributions were measured by using dual-energy X-ray absorptiometry (Hologic APEX,
discovery W (S/N 84030), version 3.0; Hologic]. REE was measured in a subset of subjects (n = 22) by using indirect calorimetry (Deltatrac; Datex). A predicted REE was also calculated for all subjects on the basis of body composition by using an equation that included both fat-free mass and fat mass (21). Psychological attitudes (22), the 3-factor eating questionnaire (23), and physical activity scores (16) were also evaluated.

**Dietary records**

At the start of the study, participants received individual counseling from a registered dietitian who also followed each individual during the different phases. A dietary intake was prescribed individually according to information obtained from dietary questionnaires (3-d recall technique) at the beginning of the study. At this time point, subjects were instructed individually in how to estimate food intake, and they completed the 7-d dietary records at baseline, 6, and 12 wk. Subjects were contacted by the same dietitian (by phone or e-mail messages) at the end of the first, second, third, and fourth weeks and in the middle of the stabilization period. The dietitian revised diary records and corrected with the participant any errors or missing data. All data were coded by the same dietitian by using a computer-software program (PROFIL Xa029, ACIM; AuditConseil).

Foods were categorized manually according to the National Nutrition and Health French Program. A total of 18 categories and subcategories were created (see Supplementary Table S1 under “Supplemental data” in the online issue).

**Gut-microbiota composition**

Fecal samples were obtained in the morning. Whole stools were self-collected in sterile boxes and stored at −20°C within 4 h, sampled as 200-mg aliquots, and stored at −80°C until analysis. Seven groups of fecal bacteria (Clostridium leptum, Clostridium coccoide, Bacteroides/Prevotella, Bifidobacterium, Lactobacillus/Leuconostoc/Pediococcus, Escherichia coli, and Faecalibacterium prausnitzii) were evaluated by using a quantitative polymerase chain reaction as described previously (24).

**Biochemical assays**

Plasma glucose was measured by using the glucose oxidase method (Beckman Coulter). Plasma insulin was determined by using an Abbott kit (Abbott). Plasma triglycerides and free fatty acids (FFAs) were measured by using DiaSys-POLES kits (DiaSys), and total cholesterol, HDL cholesterol, and LDL cholesterol were measured by using colorimetric enzymatic tests (Thermo Scientific). Leptin, adiponectin, and IL-6 were determined by using ELISA kits (R&D Systems). High-sensitivity C-reactive protein was measured by immunonephelometry on an IMMAGE analyzer (Beckman-Coulter).

**Statistical analysis**

All values are expressed as means ± SEMs. R software (version 2.14) was used for most of the statistical analyses, along with different R packages. A principal component analysis was used to explore dietary profiles on the basis of dietary terms and performed with the FactoMineR (R Package for Multivariate analysis; Harrell Miscellaneous). Genie software (version 2.0; Decision Systems Laboratory, University of Pittsburgh) was used for learning BN. Data were considered significant at P < 0.05.

**Classification of subjects by their kinetic profiles of weight changes**

The K means for longitudinal data (KmL) was thought adequate because of its ability to take into account the individual trajectories in longitudinal data. However, some subjects were not correctly classified on the basis of their weight profiles in the stabilization period (see Supplemental Figure S1 under “Supplemental data” in the online issue). The numeric distribution of figures for the identified 3 clusters as proposed by Calinski criteria did not present a satisfactory representation for adequate comparison. The combination of the KmL method (R package = KmL) and 95% CI was used to cluster subjects depending on the trajectories of their weight changes.

**Link between weight-change clusters and bioclinical and gut-microbiota variables at baseline**

Kruskal-Wallis rank-sum tests were used to assess differences when clusters were compared, whereas Wilcoxon’s rank-sum tests were used to assess differences between each set of 2 clusters as related to the nonparametric distribution of variables. Spearman’s rank test (R package = Hmisc) was used to evaluate the relations among environmental factors, food intake, and bioclinical variables at baseline and during the dietary intervention. The significance of correlations was expressed by P values, and the significance of coefficients was expressed by r values. Stratified Kruskal tests (R package = coin) were applied to test the influence of food items on body weight changes in the 3 clusters at 6 and 12 wk. Partial Spearman’s tests were used to adjust results at baseline and the correlation between weight variation and numbers of the Lactobacillus/Leuconostoc/ Pediococcus group by food items.

**BN application for identification of initial predictors of weight changes**

We constructed a BN with Genie 2.0 software (Decision Systems Laboratory) to identify predictors of body weight changes. For the learning step of the BN from the (discretized) data, we used the Gready Thick Thinning algorithm (25) from the Genie/Smile library (see online supplementary data under “Supplemental data” in the online issue for additional explanation). The BN included variables that were highly correlated to body weight changes and influenced by the dietary intervention. We selected 12 biological variables; 10 variables differed significantly in clusters at baseline (fasting insulinemia, HOMA of insulin sensitivity, HOMA-IR, QUICKI, the McAuley index, leukocytes, neutrophils, IL-6, HAM56, and Lactobacillus/Leuconostoc/Pediococcus) as shown in Table 1. Triglycerides and fasting FFAs were added to the set of knowledge information mentioned, and the cluster of weight variation (clusters) was also included to build the BN. FFA AUCs and some surrogate insulin indices that differed in the 3 clusters at baseline (n = 41) were not included in the BN because of technical problems with performing the OGTT in all subjects. Each included variable represented a node in the network.
Plasma glucose homeostasis and insulin sensitivity were assessed using the oral-glucose-tolerance test, respectively. Significance remained after correction by using the Benjamin and Hochberg method. Means within a row that do not share a common superscript letter are significantly different at the p < 0.05 level. SI is OGTT, simple index assessing insulin sensitivity derived from oral-glucose-tolerance test.

**TABLE 1**
Clinical characteristics and gut microbiota in the 3 clusters at baseline

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Sex (F, M)</th>
<th>Age (y)</th>
<th>Energy balance</th>
<th>Adiposity markers</th>
<th>Adipocyte markers</th>
<th>Waist circumference (cm)</th>
<th>Hip circumference (cm)</th>
<th>BMI (kg/m²)</th>
<th>Body weight (kg)</th>
<th>Leptin (ng/mL)</th>
<th>Fasting insulinemia (µU/mL)</th>
<th>HOMA-B (%)</th>
<th>HOMA-S (%)</th>
<th>QUICKI</th>
<th>SI is OGTT index</th>
<th>Disse index</th>
<th>McAuley index</th>
<th>SlsOGTT index</th>
<th>Matsuda index</th>
<th>Adiponectin (µg/mL)</th>
<th>Fasting FFA (mmol/L)</th>
<th>Leukocytes (giga/L)</th>
<th>Neutrophils (giga/L)</th>
<th>Monocytes (giga/L)</th>
<th>hCRP (mg/L)</th>
<th>IL-6 (pg/mL)</th>
<th>Fecal microbiota</th>
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<tr>
<td><strong>Cluster A (n = 17)</strong></td>
<td></td>
<td>15, 2</td>
<td>42.6 ± 2.6*</td>
<td>14.1</td>
<td>12.5</td>
<td>44.1 ± 3.3</td>
<td>40.2 ± 3.2</td>
<td>33.0 ± 1.0</td>
<td>89.6 ± 6.1</td>
<td>80.7 ± 6.1</td>
<td>49.6 ± 6.1</td>
<td>5.16 ± 0.08</td>
<td>8.52 ± 1.1*</td>
<td>11.07 ± 1.1*</td>
<td>0.01*</td>
<td>1.28 ± 0.3*</td>
<td>0.13 ± 0.1</td>
<td>2.39 ± 0.39*</td>
<td>0.3 ± 0.1*</td>
<td>7.77 ± 0.99*</td>
<td>8.56 ± 1.02*</td>
<td>13.31 ± 1.11</td>
<td>14.81 ± 1.91</td>
<td>0.67</td>
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<td><strong>Cluster B (n = 15)</strong></td>
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<td><strong>Cluster C (n = 17)</strong></td>
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1. n = 49 subjects. LDL values were calculated by using Friedewald's formula. QUICKI = 1 + [log(I0) + log(gly0)]; Disse index = 12 × [2.5 × (HDL cholesterol + total cholesterol) – FFA0] – I0; McAuley index = e0.25 – 0.28I0 – 0.31 I0I30 + I90 + I120; SlsOGTT index = 1 + [log(I0 + I30 + I90 + I120) + log(gly0 + gly30 + gly90 + gly120)]; and Matsuda index = 10^5 × [gly0 × I0 × mean(gly0,30,60,90) × mean(I0,30,60,90,120)^0.5], where gly0, I0, FFA0, and TGG0 represent fasting plasma glucose, insulin, FFAs, and triglycerides, respectively; gly30,60,90,120 and I30,60,90,120 are glycemia and insulinemia at 30, 60, 90, and 120 min after an oral-glucose-tolerance test, respectively. *Significance remained after correction by using Benjamin and Hochberg. Means within a row that do not share a common superscript letter are significantly different at p < 0.05 on the basis of Wilcoxon’s rank-sum test. FFA, free fatty acid; HOMA-B, homeostasis model assessment to estimate pancreatic insulin secretion; HOMA-S, homeostasis model assessment to estimate insulin sensitivity; hCRP, high-sensitivity C-reactive protein; QUICKI, quantitative insulin sensitivity check index; SlsOGTT, simple index assessing insulin sensitivity derived from oral-glucose-tolerance test.

2. Kruskal-Wallis rank-sum test was used to give the variance in 3 clusters at each time point.

3. Mean ± SEM (all such values).

4. n = 41 subjects.

5. Cell number marked by antibody anti-HAM56 + the adipocyte number.

6. Fecal microbiota are expressed as mean of the log10 value ± SEM of normalized data and calculated as the log number of targeted bacteria minus the log number of all bacteria.
Because the BN is better performed after discretization, we have chosen a statistically justified heuristic method (Chi-Merge) to supervise the discretization on random data; a very high threshold must be set to avoid creating too-many intervals. First, we used the Chi-Merge method (R package = dprep) and set \( \alpha = 0.01 \) (the highest threshold) so that 13 bioclinical variables (except the cluster of weight variation, as already discretized) could be discretized in \( \geq 2 \) states and constrained by the 3 clusters of weight loss. Second, the BN with the discretized variables was learned by using the greedy BDeu method.

We used the data collected at baseline from all subjects \( (n = 49) \) after setting certain knowledge information, such as the dependent relations in surrogate insulin-resistance indices and plasma glucose, insulin, and triglyceride concentrations. Because we had one node (clusters) including 3 different clusters as the target (T) and 13 discretized variables \( (X) \), the posterior probability \( P (T = j \mid X_1, \ldots, X_n) \) represented the probability of a profile \( (X_1, \ldots, X_n) \) to be classified in the cluster \( j \) \((j \text{ in A,B,C})\). Then the predicted cluster of the profile was the value of \( j \) that maximized \( P (T = j \mid X_1, \ldots, X_n) \). This method is known as the maximum a posteriori (MAP) principle. According to the learned structure and the MAP principle, the learned BN and prediction of the cluster are exploitable. An interpretation of the structure of the BN might be misleading because a common error is to consider the arrows as causal relations although they indicate conditional dependent relations. Precisely, the structure represents the conditional independences in variables by using a criterion called d-separation (26).

**Validation of classifier**

The predictive accuracy of the models was tested with the same set of data used to develop the models, which could have led to an overestimation of the true predictive accuracy. A common process of classifier validation is to separate the data into a test set and a training set and to learn the model with the training set and evaluate the performance on the test set. This usual approach was not appropriate because of the relatively small data set. Nevertheless, we addressed this concern by using validation approaches of the classifier, first by using leave-one-out crossvalidation (LOOCV) (27), which used all the data minus one subject for repeated times, and second, with the area under the receiver operating characteristic (ROC) curve to evaluate the classification performance of the produced model. Three ROC graphs were built with LOOCV data, one for each cluster (against the union of the 2 others). We also tried to examine the relevance of putative known and expected relations in variables that could be automatically learned from the data.

**Comparison of BN to other classical methods of prediction**

We have compared the BN approach to 3 other standard statistical machine learning methods, both with the 13 pre-selected variables and the resulting 4 variables that constructed the Markov blanket. We tested the naïve Bayes (28) on discretized data and the multiclass (3 classes) logistic regression (29) on both discretized and original continuous data.

**RESULTS**

**Subject classification by kinetic profile of weight changes during dietary intervention**

Before starting the clusterization of subjects, we verified that there was no significant difference in body weight between men and women at baseline; also, there was not a significant difference for changes in body weight. Moreover, the adherence to dietary recommendations was similar between men and women (see Supplemental Figure S2 under “Supplemental data” in the online issue). Therefore, men and women were grouped in the subsequent analysis.

Three groups (clusters A, B, and C) were classified according to their weight profile during the nutritional intervention (Figure 1). Cluster A subjects \((n = 17)\) had the best weight loss during energy restriction and continued weight loss during the weight-maintenance period (compared with basal values: mean \( \pm SEM \) of \(-7.6 \pm 0.3\% \) at 6 wk and \(-10 \pm 0.4\% \) at 12 wk). However, subjects in cluster C \((n = 17)\) showed an opposite profile of less weight lost during the first 6 wk \((-4.4 \pm 0.2\%\) and more weight regained during weight maintenance (a total of \(-1.5 \pm 0.3\%\) compared with in the other groups (see Supplemental Table S2 under “Supplemental data” in the online issue). The remaining 15 subjects were grouped into cluster B and characterized by weight loss \((-5 \pm 0.3\%)\) at 6 wk and weight stabilization during the second phase \((-5 \pm 0.2\%)\).

As expected, we observed differences in kinetic changes of adiposity and metabolic and inflammatory variables in the 3 clusters during the dietary intervention (see Supplementary Table S2 under “Supplemental data” in the online issue). Compared with the other clusters, subjects in cluster A had a better reduction in waist and hip circumferences, adipocyte diameter, and leptin and a better increase in insulin sensitivity and fasting plasma FFA. Although the overall food intake (macronutrient and calorie intakes) did not differ in the 3 clusters, they did differ qualitatively in food consumption. Cluster C consumed the highest amount of starch and oils but the lowest amount of raw vegetables and proteins (see Supplemental Table S1 under “Supplemental data” in the online issue), particularly during the

![FIGURE 1. Clusters of mean (±SEM) weight loss during the weight-loss/weight-maintenance dietary program. Weight loss at each time point is presented as a percentage of baseline values. The black line shows the profile of weight loss for cluster A (17 subjects who lost weight even during the maintenance period), the dark gray line represents cluster B (15 subjects who correctly followed the protocol), and the light gray line represents cluster C (17 subjects who lost less weight and regained this weight during the weight-maintenance period).](Image)
weight-stabilization period. Cluster C also consumed more dairy products, cooked fruits, and some minerals (see Supplementary Table S1 under “Supplemental data” in the online issue). However, differences in weight variation and bioclinical markers at 6 and 12 wk in the 3 clusters persisted after adjustment by food items by using stratified Kruskal tests.

Differences in metabolic and inflammatory variables and gut-microbiota groups in 3 clusters at baseline

We further searched for baseline differences in the 3 clusters that could be candidate predictors of different weight trajectories. Sex, age, or adiposity markers were similar as were physical activity and psychological scores (see Supplemental Table S3 under “Supplemental data” in the online issue). Of importance, total calorie intake, nutrients, and food items were similar in the 3 clusters (Table 1; see Supplementary Table S3 under “Supplemental data” in the online issue). We observed significant differences in glucose homeostasis, lipid variables, inflammatory markers, and gut-microbiota components (Table 1). Fasting insulinemia and insulin resistance were higher in cluster C than clusters A and B (Table 1). The increase in insulin resistance was illustrated by the modification of surrogate indices of insulin resistance evaluated at fasting as homeostasis model assessment to estimate insulin sensitivity, QUICKI, or the McAuley index. The AUC of FFAs was the lowest in cluster C. In addition, plasma leukocyte and neutrophil numbers as well as plasma IL-6 concentrations were the highest in cluster C (Table 1). The increase in low-grade inflammation was also associated with an increase in HAM56+–stained cells in scAT, which reflected the accumulation of inflammatory cells (mostly macrophages) (Table 1). Cluster C had the highest number of the Lactobacillus/Leuconostoc/Pediococcus group but not of the other 6 measured fecal bacterial groups. Results remained significant even if the 8 men were removed from the analysis (data not shown).

Relation between metabolic markers at baseline and weight changes during the dietary intervention

We assessed whether metabolic, inflammatory, and gut-microbiota components at baseline were quantitatively associated with weight variations. Weight regain during the stabilization period correlated positively with baseline values of fasting plasma insulin (see Supplemental Table S4 under “Supplemental data” in the online issue), insulin resistant indices, and inflammatory markers (such as leukocytes, neutrophils, and IL-6), whereas weight regain was negatively correlated with insulin sensitivity indices and the AUC of FFAs. By contrast, weight variation did not correlate at any time point with nutrients and food intake at baseline. The number of the Lactobacillus/Leuconostoc/Pediococcus group at baseline was also positively correlated with weight regain. These correlations persisted after adjustment by dairy products at 6 wk (P = 0.05, partial r = 0.27) and 12 wk (P = 0.002, partial r = 0.41) but disappeared when adjusted for the quantity of starch products. Weight loss during the weight-loss period (changes from baseline to week 6) did not correlate with the different variables at baseline.

BN classifier identified candidate predictors of weight loss

The structure of the learned BN is shown in Figure 2. To use this BN as a classifier, we needed to compute the probability of target-variable clusters (indicating the probability of belonging to cluster A, B, or C) conditionally to the values of the 12 selected variables (see BN application in the statistical analysis section). The most interesting part in the topology of the BN was that clusters depended only on the following 4 variables that constituted its Markov blanket: its parent (HAMP56) and its children (IL-6, leukocytes, and fasting plasma insulin). Thus, although the probability of belonging to a cluster may have a priori depended on all 13 variables [noted P (clusters | the 13 variables)], according to the Markov property of the Markov blanket, it depended, in our case, on only the 4 identified variables [noted P (clusters | fasting plasma insulin, plasma leukocytes, IL-6, and HAMP56)]. The learned BN provided other relevant and expected information on associated variables (Figure 2; see Supplementary Material, page 12, under “Supplemental data” in the online issue). Figure 2 does not include the Lactobacillus/Leuconostoc/Pediococcus group at baseline because this variable was conditionally independent of any other variables (ie, not connected in the graph).

To test our model, for each subject, we estimated the value of clusters given the different states of these 4 variables determined by using the Chi-Merge method (see Supplemental Table S5 under “Supplemental data” in the online issue) and the MAP (see Supplemental Table S6 under “Supplemental data” in the online issue). Thirty-seven of 49 subjects were correctly
classified with a probability of 75.5%. Of note, insulinemia, plasma leukocytes, IL-6, and HAM56 in adipose tissue had the following values: 5 (8.6–20 μU/mL), 2 (6.27–9.8 giga/L), 2 (1.71–10.57 pg/mL), and 2 (3.3–9.8 numbers/field), respectively, which allowed us to compute that the probability of belonging to cluster C was 94%. Thus, when a subject at baseline had insulinemia >8.5 μU/mL, leukocyte number >6.5 giga/L, IL-6 concentration >1.71 pg/mL, and HAM56 >3.3 numbers/field, this subject might have had a 94% chance of regaining body weight after dietary weight loss as witnessed in cluster C subjects (see subject 5 in Supplementary Table S6 under “Supplemental data” in the online issue).

Validation test of identified classifier

With the analysis of the structure of the BN, we retrieved known and expected relations in variables that have been automatically learned from the data (Figure 2). Although this retrieval supported our results, we nevertheless used validation approaches to test the relevance of the BN-identified classifier. First, the result of LOOCV was almost exactly the same as learning all data (see Supplementary Table S6 and Supplementary Figure S3 under “Supplemental data” in the online issue). Second, ROC curves showed a good performance of the 3 binary one-cluster classifiers (AUC >0.88). In addition, the MAP multiclass classifier showed a good ratio between sensibility and specificity (Figure 3), and this classifier guaranteed an existing and unique predicted cluster for each profile. ROC curves when learning all the data and those after the LOOCV analysis were also comparable (see Supplementary Figure S3 under “Supplemental data” in the online issue).

Comparison of BN to other typical methods of prediction

When we ran the experiments with the 4 variables shown by using the BN, we obtained the following results: 1) naive Bayes on the discretized data and LOOCV accuracy of 75.51%, 2) logistic regression on the discretized data and LOOCV accuracy of 69.39%, and 3) logistic regression on the continuous data and LOOCV of 51.0%. ROC curves are presented in the supplementary material (see Supplemental Figure S4 under “Supplemental data” in the online issue). When we ran the results with the 12 discretized variables, results were as follows: naive Bayes, train accuracy of 85.7%, and LOOCV accuracy of 77.55%.

FIGURE 3. Receiver operating characteristic curves of learning leave-one-out cross validation data. Receiver operating characteristic curves were generated for each cluster in turn by learning the leave-one-out cross validation data. Each cluster was plotted against the other 2 clusters [clusters A (A), B (B), and C (C)]. The gray point in each graph represents the threshold of the MAP multiclass classifier. MAP, maximum a posteriori.
The results were apparently better than with the 4 variables (LOOCV of 75.51%), but the difference in the performance was not highly significant with 75.51% accuracy, 37 patients (of 49) correctly predicted, compared with 77.55% accuracy that corresponded to 38 correctly classified patients. ROC curves are presented in the supplementary material (see Supplemental Figure S5 under “Supplemental data” in the online issue). Finally, with the multiclass (3 classes) logistic regression, we obtained a model with 126 variables and an LOOCV accuracy of 69.39%, which was not better than for the logistic regression with 4 Markov-blanket variables. When we attempted to reduce the model’s complexity by the $\ell_1$-$\ell_S$ penalty term (30), we could not ameliorate the performance.

**DISCUSSION**

We performed a 12-wk dietary intervention of 6 wk of weight loss and 6 wk of weight stabilization in obese subjects to search for predictors of weight-variation profiles. With the use of a method of clusterization, we showed 3 main profiles, and subjects in cluster C were less responsive to the dietary intervention and regained more weight during the stabilization period than did the other 2 clusters.

Subjects in cluster C, who lost less weight and regained most of it, had, at baseline, more insulin resistance and more systemic and adipose tissue inflammation than did the other 2 groups. Subjects in cluster C had lower excursions of FFAs and higher amounts of one component of gut microbiota. The number of the *Lactobacillus/Leuconostoc/Pediococcus* group was the highest in cluster C, and the number of this bacterial group at baseline was also correlated positively with weight regain during the weight-maintenance phase.

Individual differences in the response to dietary interventions arise from complex phenotypes modulated by many factors. In the current study, we used, for the first time to our knowledge, an original approach with BNs to test the relevance of the association shown and whether the combination of gut-microbiota components and biological and environmental variants could improve prediction.

Although overall global calorie intake was similar in the 3 clusters, there were slight qualitative differences. When we took the whole study period into consideration, subjects in cluster C ate fewer raw vegetables but more starch and oils. These changes were correlated with a less-efficient amelioration of adiposity markers. However, these qualitative differences did not fully explain the different weight trajectories because differences in the 3 clusters remained after adjustments for these food items.

These findings suggested that other factors may explain the responses. We observed that the combination of metabolic and inflammatory variables contributed significantly to the relative resistance to the dietary intervention in cluster C. Subjects in cluster C had increased insulin resistance on the basis of measures of surrogates of insulin sensitivity. Although we did not evaluate insulin sensitivity by using a clamp technique, the use of several surrogates of insulin resistance at the fasted state and during the OGTT could be considered relevant, especially at this level of BMI (20, 31). Nevertheless, the use of measures of insulin sensitivity to predict the outcome of a dietary intervention has proved controversial in different populations. In a long-term (3.5 y) weight follow-up study (and not an energy-restricted weight-loss program), insulin resistance was shown to be associated with reduced risk of weight gain in nondiabetic Pima Indians (32). In other studies in similarly overweight or obese populations, baseline fasting plasma insulin was not shown to predict weight loss induced by energy restriction in healthy obese women (33) or weight maintenance (10). Although reduced insulin secretion during the OGTT was shown as a predictor for weight regain in Pima Indians (34), increased insulin secretion was shown as a predictor of weight maintenance in a European population in another study (10). The reasons for these discrepancies are not known, but all of these studies were performed in populations of different origins and with different designs relative to the nature and duration of the intervention.

In our study, the insulin burst in response to the OGTT was not different in cluster C subjects, who had higher insulin resistance in the fasting state. Of importance, the BN used here filtered the main information so that low plasma insulin was identified as an important predictor of weight loss and maintenance. Indeed, although insulin-resistance surrogates differed in the 3 clusters at baseline, it was the combination of plasma insulin and inflammatory markers that could predict the weight trajectory. Less excursions of FFAs during the OGTT were shown in cluster C subjects at baseline. Indeed, during the energy-restricted dietary programs, plasma FFA increased, which suggested increased lipolysis (35). The decreased FFA excursion in cluster C indicated less lipolysis, which might have been related to the anti-lipolytic effect of insulin in these subjects with higher insulin concentrations. A defect in other factors that stimulate lipolysis could have also been implicated in these results. FFA excursions in the current study could not be excluded as a predictor; unfortunately, this factor was not included in the BN analysis because it was not obtained in all the subjects.

The triglyceride concentration could also predict weight loss. Noakes et al (36) showed that obese subjects in the top 50% of blood triglyceride concentrations at baseline lost more weight with a hypocaloric high-protein diet than a hypocaloric, high-carbohydrate diet. The authors noted that this was because of the fact that subjects who had high triglyceride concentrations were insulin resistant. In our study, triglyceride concentrations at fasting were not associated with the outcome of the dietary intervention. Moreover, the 2 circulating adipokines leptin and adiponectin, which were suggested previously to be linked to the outcome in dietary interventions (5, 37), were not shown to be relevant predictors that explained the 3 weight trajectories. In a 2-y dietary trial (5) that included weight-loss and weight-maintenance phases, higher baseline adiponectin and a greater reduction in leptin concentrations during the weight-loss period were the main predictors for successful weight maintenance. However, predictors for the 2 periods were separated, which was not the case in the current study in which the evolution of adiponectin differed in the 3 clusters despite similar values at baseline. Adiponectin concentrations decreased in cluster C but increased in the other 2 clusters, which had better responses at the end of the whole dietary program.

Because of the complexity of obesity and metabolic disorders, a variety of other factors might contribute to responses after a dietary intervention. Gut microbiota have been suggested as involved in different pathophysiologic pathways of weight gain such as energy harvest, lipid metabolism, gut-barrier leaking, and inflammation (11). We hypothesized that measures of the
microbiota could be useful for prediction of weight changes. The number of the Lactobacillus/Leuconostoc/Pediococcus group was highest in cluster C, and the number of this bacterial group correlated positively with weight regain. The consumption of starchy products, and not the consumption of dairy, seemed to influence these correlations. This finding might be the reason why the Lactobacillus/Leuconostoc/Pediococcus group was not in the Markov blanket of BN because this bacterial group seemed to be directly linked to food intake rather than being a good predictor of weight variation. Our results could not contribute to the actual debate concerning the role of Lactobacillus in weight gain (38) because we detected a bacterial group, not just Lactobacillus. Of note, when we used the BN, this group was not a relevant predictor of weight trajectory. With the development of metagenomic approaches, it will be important to test whether other bacterial genes or species could be considered relevant predictors.

Previously used methods could not show the complex interactions in variants and predictors and the relevance of the information. With the use of the BN approach, which included variables that were highly correlated to body weight changes and influenced by the dietary intervention, we were able to identify potential predictors. Bayesian analyses predicted that subjects with the combination of high fasting plasma insulin, plasma IL-6, leukocyte number, and adipose tissue HAM56 at baseline would not have the best weight profile. The BN model allowed for the identification of conditional independent relations in variables and filtered the most relevant information and calculated the probability of prediction. However, the accessible information was insufficient to characterize perfectly the different classes because only the main pertinent variables rather than all variables were included in BN analyses. Of 49 participants, 37 participants (75.5%) were correctly predicted according to their cluster category by relying on 4 predictors only. These 4 predictors have reasonable functional interactions because obesity and insulin resistance have a low-grade inflammation component. Chronic tissue inflammation is recognized as an important cause of obesity-induced insulin resistance (39–41).

When we compared the proposed approach to state-of-the-art methods, we concluded that the approach used in the current study was, to our knowledge, the best method. The logistic regression is often considered to be more efficient than the naive Bayes. Researchers have proposed that initial continuous data contain more information than do discretized data. We suggest that, in our case, the discretization added some information, and at least the model of the logistic regression with the discretized data was richer (33 variables for the model with 4 variables) than was the logistic regression with the continuous data (8 variables). In addition, some theoretical (42) and practical evidence has suggested that logistic regression is more efficient than the naive Bayes only asymptotically, but in cases when the number of observations is relatively small, the naive Bayes performs better.

Finally, an improvement of the picture of how inflammation modulates metabolism may provide new opportunities for using antiinflammatory strategies to correct metabolic consequences of excess obesity (43). A remaining open question is whether the use of healthy diets (ie, that contain components linked to decreased systemic or tissue inflammation) or pharmacologic approaches could be relevant in dietary-induced changes in weight trajectory of subjects with high inflammation and insulin resistance. Additional interventional studies are needed to evaluate the effect of these strategies.

In conclusion, with consideration of the complex biological interactions between biomarkers, we suggest that the use of a network modeling combining 4 biological variants at baseline can identify potential causal predictors of successful weight loss and maintenance in response to a dietary intervention. We note that the current study was performed in a limited number of moderately obese (majority of women) subjects during a short-term dietary intervention, with a special type of hypocaloric diet (slightly high in proteins and with soluble fibers). Additional approaches are needed to elucidate the relevance of combining biomarkers to predict trajectories in different populations during short- and long-term weight-loss and maintenance programs.

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REFERENCES


